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Estimation of Salivary Calcium and Phosphorus in Children with different caries status – A Cross-Sectional observational study

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ABSTRACT

Background and Objectives

Early Childhood Caries (ECC) is a chronic disease of childhood affecting the primary dentition. ECC begins early in life and may impact the quality of life of a child. It is also termed as nursing caries or baby bottle tooth decay. It has a multifactorial etiology and salivary parameters are considered as one of the key etiological aspects of ECC. The current research was done to assess the level of salivary phosphorus and calcium in children with ECC, S- ECC(severe ECC) as well as caries-free. The main objective of the study is to determine the correlation between calcium and phosphorus level and to compare it with the three groups of children with different caries experiences.

Materials and Methods:

It is cross-sectional observational research carried out at Saveetha Dental College and Hospitals. Children between 3-6 years were examined and 20 caries-free, 20 children with ECC as well as 20 children with S-ECC were recruited for the study. The examination and sample collection were done by a single qualified Pediatric dentist. The samples were collected and analysed for salivary calcium and Phosphorus.

Results:

The results demonstrated that the statistically substantial variations in salivary calcium and phosphorus levels among caries-free, ECC as well as S-ECC

Conclusion:

1. There was a substantial variation in salivary calcium levels between caries-free, ECC as well as S-ECC children.
2. There is a substantial variationin salivary phosphorus level between caries-free, moderate ECC as well as S-ECC children.

Key Words: Saliva, Salivary Calcium, Salivary Phosphorus, Early Childhood Caries

Introduction

Despite continuous research for more than a century, ECC still remains a prevalent disease in children. ECC begins early in life and may influence the value of life of the child. ECC is defined as “the presence of one or more decayed (non-cavitated or cavitated lesions), missing teeth (due to caries), or filled tooth surfaces in any primary tooth in a child 71 of months age or younger”. Any smooth surface caries symptom in a child less than 3 years of age is considered as ECC. From the age of 3 -5, the existance of one or more decay, filled surfaces (dmfs), or missing teeth in main anterior teeth is indicative of S-ECC. Similarly, dmfs score of ≥6 (age 5), ≥5 (age 4), or >≥4 (age 3), surfaces show S-ECC. [1,2] Children require healthy teeth and chewing ability while the transition from a weaning diet, as dietary intake, is important to maintain the nutritional status of children. [3,4] ECC is associated with pain and swelling and can affect the chewing ability of children, thereby indirectly affect

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the nutritional status of the children. Hence, it is of paramount importance to treat ECC at an earlier stage to prevent negative health impacts.

Saliva plays a vital role in keeping the integrity of teeth and oral structures. Whole saliva is composed of 10% of secretion by gingival crevicular fluid and minor salivary glands and 90% of secretion from major salivary glands (sublingual, submandibular and Parotid,). [5] Saliva is consisting of 99 percent water & less than 1% of inorganic and organic components. Though these inorganic and organic contents are less than 1%, they play an imperative role in preserving the stability of teeth and preventing the remineralization process. The functions of saliva include aggregation and removal of microorganisms, neutralize acids through buffering action, elimination of food debris, anti-microbial action, and participate in acquired enamel formation. [6,7]

Calcium and phosphate ions maintain the saliva in the supersaturated state with respect to hydroxyapatite crystals of enamel and maintain the integrity of the tooth. [8] If this environment is maintained, it will inhibit the demineralization process and enhance remineralization. In addition, these ions influence the calcium hydroxyapatite dissolution or precipitation, the main inorganic component of hard dental tissues. [9] Phosphorus and Salivary Calcium play a pivotal role in enamel maturation after eruptive and helps in rehardening softened, demineralized enamel. [10,11] Demineralization occurs when there exists a disproportionate in the mineral content between the tooth and the oral environment. During these series of events, the enamel crystal is dissolved by the acids produced by the micro-organisms and leads to loss of these ions in the saliva leading to demineralization of the tooth. Low level of salivary calcium and phosphate levels affects the equilibrium in demineralization & remineralization process and ultimately leads to caries development. Hence it explains the importance of salivary calcium and phosphorus ions in maintaining and preserving the tooth integrity from demineralization. Jenkins et al expressed that, the salivary level of calcium in a healthy person is 6mg/dl in stimulated saliva and “5.8mg/dl (2.3 to11.3mg/dl) in resting saliva” [5] Parotid saliva includes more phosphate and fewer Ca+2 ions than mixed saliva. In the contrast, the Zn+3, Mg+2, and Ca+2 levels in the mixed saliva are considerably greater than the parotid saliva.

Numerous researches were carried out to explore the relationship between salivary calcium and phosphorus level and ECC. Turtola et al and Elizarova and Petrovich reported an increase in salivary calcium in children with increased caries activity, [12,13] whereas a pilot study done by Aruna et al reported a rise in salivary calcium levels in caries-free children. [14] Studies had reported a rise in salivary phosphorus levels in caries active children. [15,16] Since wide disparity exists in the results obtained from the above-mentioned studies, the goal of this research is to assess the level of salivary calcium & phosphorus in children with ECC, S-ECC, and caries-free. The main objective of the “study is to determine the correlation between” calcium as well as phosphorus level and to compare it with the three groups of children with different caries experiences.

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Materials and Methods

Type of Study

The present study is a cross-sectional observational study. Children between the ages of 3-6 years, who reported to the "Department of Pediatric and Preventive Dentistry", Saveetha Dental college were involved in the study.

Ethical Approval

This study "Estimation of Salivary Calcium and Phosphorus in Children with different caries status – A Cross-Sectional observational study" has been authorized through the "Ethics Committee" of the "Saveetha Dental College and Hospitals". The study protocol was recorded by an informed approval by the "Ethics Committee" of the "Saveetha Dental College and Hospitals". 20 children with ECC and 20 children with S-ECC were selected based on dmft index and 20 caries free children were recruited for participation in this study

Inclusion Criteria

Children of both genders between 3 - 6 years old
Parents who accept to participate in the research with an informed consent

Exclusion Criteria

Uncooperative children who don'tallow thecollection and/or examination of saliva
Children with pharmacological treatment and/or systemic diseases

Children have divided 3 groups; Group I – Caries free (dmfs=0), Group II- ECC (dmfs=1-3), and Group III- S-ECC (dmfs>3) with 20 children in each group based on AAPD Guidelines. A total of 60 children with age group of 3-6 years were randomly selected, who reported Saveetha Dental College and Hospitals. After obtaining informed written approval from the parent/guardian the children were enrolled in the research.

Clinical Examination

The caries status was reported based on WHO Criteria using dmfs (missing, decayed, filled surfaces) index. [17] The examination was done under dental chair light utilizing a sterile probe and mouth mirror. The dental examination was completed within 10 min and an assistant recorded the clinical findings.

Saliva Collection

To avoid circadian variation, the salivary samples were obtained between 10.00 to 11.00 am. The children were refrained from eating or drinking anything 1 hour before the salivary sample collection to prevent contamination. [18-20] the children were instructed to clean their mouth to remove any food debris present. Saliva was obtained according to the methodology given by Wu et al. [21]. The child is allowed to sit in a "well-ventilated" along with well-lit environment. The children were asked to keep their head at 45ºC, hold the sterile tube with one hand. The saliva was permitted to drop into the tube and allowed till sufficient for analysis

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without measuring the froth the quantity. About 5ml of unstimulated saliva has been obtained from the children in a plastic rube.

The salivary samples were transferred to the laboratory immediately to estimate the amount of salivary calcium and phosphorus. The salivary calcium was estimated using the Spin react colorometric kit and salivary phosphorus was estimated using the Spinreact phosphorus-UV kit. Salivary Calcium reacts with neutral pH of "Arsenazo III (1,8-Dihydroxy-3,6-disulpho-2,7- naphthalene-bis (azo)-dibenzenearsonic acid)", resulting in a complex of blue-colored. The color intensity is related to its calcium level of the salivary sample. [22-24] The inorganic phosphorus reacts in "ammonium molybdate" in an acid medium to generate a phosphomolybdate complex of yellow color. The strength of the produced color is related to the sample content of inorganic phosphorus. [25,26]

Statistical Analysis

Each data has been entered and analysed applying the software SPSS 20.0. One-way ANOVA was done to determine the difference between salivary calcium and phosphorus between the groups. "Post-hoc Tukey" test was done to measure the intergroup significance. A "P value of < 0.05" was measured as statistically "significant" and $P < 0.001$ was deemed as statistically "highly significant".

Results

The mean & standard deviation value for salivary calcium is depicted in Table 1. In "Group I (caries-free) the mean" levels of calcium were found to be 2.19+/-0.52 mg/dl, Group II (ECC), the mean concentrations of calcium were 2.69+/-1.11 mg/dl and Group III (S-ECC), the mean concentrations of calcium were 3.46 +/-1.05 mg/dl. The mean salivary calcium levels in the study groups ($P < 0.05$) were substantially different. Post hoc Tukey test revealed that Group III had a considerably greater mean salivary calcium level than that of Groups I and II. However, the mean salivary calcium level from Group I & II was not significantly different. (Table 2)

The mean & standard deviation value for salivary phosphorus is depicted in Table 3. In Group I (caries-free) the mean levels of calcium was found to be 5.45+/-0.89 mg/dl, Group II (ECC), the mean concentrations of calcium were 7.83 +/-1.39 mg/dl and Group III (S-ECC), the mean concentrations of calcium were 7.86 +/-2.72 mg/dl. The mean salivary calcium levels in the study groups ($P < 0.05$) were substantially different. "Post hoc Tukey" test revealed that the mean salivary calcium in Group III was substantially greater than that in Groups I and II. The mean salivary calcium levels of Group I and Group II was significantly different. However, no substantial variation was evident in Group II & III. (Table 4)

Discussion

ECC remains as a main public health issuebecause of its high prevalence in the world.[27] Salivary components namely inorganic and organic components play an imperative role in protecting teeth and surrounding soft tissues. [28,29] Though several etiological factors namely, microbial challenge, improper feeding patterns, and improper oral hygiene measures play a role in the initiation of ECC, they alone are not sufficient to initiate ECC. So other salivary parameters also play a role in ECC progression and initiation. Saliva has an inherent ability to buffer strong acids & bases and gives phosphate and calcium ions necessary for the remineralization of teeth.

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The balance in remineralization, as well as the demineralization cycle and the amount of this ion, supersaturated in the saliva, is of paramount importance to prevent dental caries.

The Levels of Salivary Calcium in our study were higher between children with Severe ECC than those of children with ECC as well as caries free children. The mean salivary calcium level of Group III is substantially greater than that of Groups I and II. However, there were no substantial variations between the mean salivary calcium levels of Group I & II. The result was according to the study done by Shahrabi M et al. [30] the author reported an increase in salivary calcium level in severe caries children, but it was not statistically important. Similarly, Turtola et al and Elizarova and Petrovich et al. reported an increase in salivary calcium levels in children with increased caries activity. [12,13] On the contrary, Aruna et al [14] and Jolly R et al [31] reported a rise in salivary calcium levels in caries-free children. A study done by Vocal, Naujoks & Brudevo reported the possible reason for the increase in calcium levels in caries active children. [32] The possible reason could be, in caries-active individuals, an increased level of calcium in the saliva may be caused by a high relative amount of submandibular saliva secreted. Another possible explanation could be a release of calcium from demineralized tooth, thereby increasing salivary calcium levels. [33,34]

The Salivary Phosphorus Levels in our research were greater among children with Severe ECC than ECC & caries free children. The mean salivary calcium levels of group III were substantially greater than those of group I and II. The mean salivary calcium levels of Group I & II were significantly different. However, no major difference was evident between Group II & III. The outcomes of the research were similar to the research done by Kaur A et al and Mahajana S et al, the authors reported an increase in salivary phosphate level in caries active than caries free children. [15, 16] On the other hand, Shahrabi M et al reported a rise in the level of salivary phosphorus in caries-free children. [30] In addition, Bagherian A and Abbas MJ reported no major variation in salivary phosphorus levels in children with and without ECC. [35, 36]

Limitation

The limitation of the study includes a smaller sample size. Hence further studies are required in near future with a larger sample size and children of the same socioeconomic level, to have a more uniform and representative sample.

Conclusion

The following conclusion may be formed in the light of available evidence.

1. There is a substantial variationin salivary phosphorus levels among caries-free, ECC as well as S-ECC children.
2. There was a substantial variationin salivary calcium levels between caries-free, ECC as well as S-ECC children.

References

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[1]American Academy of Pediatric Dentistry. (2011). Policy on Early Childhood Caries (ECC): classifications, consequences, and preventive strategies. *Pediatr Dent*, 30(7 Suppl), 40–43.

[2] Kawashita Y, Kitamura M, Saito T. (2011). Early childhood caries. *Int JDent*, 725320.

[3] Çolak H, Dülgergil Ç, Dalli M, Hamidi M. (2013). Early childhood caries update: A review of causes, diagnoses, and treatments. *J Nat Sci Biol Med*,4(4), 29–38.

[4] Masumo R, Ndekero T, Carneiro L. (2020). Prevalence of dental caries in deciduous teeth and oral health-related quality of life among preschool children aged 4–6 years in Kisarawe, Tanzania. *BMC Oral Health*,20(1),1–10

[5] Jenkins NG, Lichter P, Muir J, Richardson OJ, and Feldman B (1978). *ThePhysiology and Biochemistry of the mouth*, 4th Ed.,284-358.

[6] Dawes C. (2008). Salivary flow patterns and the health of hard andsoft oral tissues. *J Am Dent Assoc*, 139, 18S–24S.

[7] Hicks J, Garcia-Godoy F, Flaitz C. (2003). Biological factors in dentalcaries: role of saliva and dental plaque in the dynamicprocess of demineralization and remineralization (part 1).*J Clin Pediatr Dent*, 28(1), 47–52.

[8] Garcia-Godoy F, Hicks MJ. (2008). Maintaining the integrity of theenamel surface: the role of dental biofilm, saliva, and preventiveagents in enamel demineralization and remineralization.*J Am Dent Assoc*, 139, 25S–34S.

[9] Anderson P, Hector MP, Rampersad MA. (2001). Critical pH in resting and stimulated whole saliva in groups of children and adults. *Int J Pediatr Dent*, 11, 266-273.

[10] Nikiforuk G. (1985). Understanding dental caries - Etiology and mechanisms, Basic and Clinical Aspects. Vol. 1: 1st ed. New York: Karger, 236-260.

[11] Mandel ID. (1987). The functions of saliva. *J Dent Res*. 66(Spec Iss), 623-627

[12] Turtola L. (1978). Dental caries and its prevention. *Pro Finn Den Soc*, 74:36-7.

[13] Elizarora VM. Petrovich IU. (1997). Ionized calcium in the saliva of children with multiple caries. *Stomatologia*, 76, 6-8.

[14] Aruna S, Meenakshi B, Rama KV, Valarmathi S. (2020). Salivary levels of calcium and phosphorus in children with and without early childhood caries: A pilot study. *SRM J Res Dent Sci*, 11(2), 72-5

[15] Kaur A, Kwatra KS, Kamboj P. (2012). Evaluation of non-microbial salivary caries activity parameters and salivary biochemical indicators in predicting dental caries. *J Indian Soc Pedod Prev Dent*, 2012, 30(3):212.

[16] Mahajan S, Suneja B, Kaur P. (2017). An attempt to correlate biochemical parameters in saliva with dental carries in children of two different age groups: A comparative study. *Int J Oral Health Sci*, 7(2), 96.

[17] Orlando, Lucia, Marcoeli. (2006). Dental caries in children that participated in a dental programme providing mother and child care.*J. Appl. Oral Sci,* 14(1), 53-60.

[18] Anderson P, Hector MP, Rampersad MA. (2001). Critical pH in resting and stimulated whole saliva in groups of children and adults. *Int J Pediatr Dent,* 11, 266-273.

[19] Dezan CC, Nicolau J, Souza DN, Walter LRF. (2002). Flow rate, amylase activity, and protein and sialic acid concentrations of saliva from children aged 18, 30, and 42 months attending a baby clinic. *Arch Oral Biol*, 47, 423-427.

[20] Soderling E, Tenovuo JO. (1989). Practical aspects of salivary analyses chapter 1: in "Human Saliva: Clinical Chemistry and Microbiology".Vol.1, C.R.C. Press, Florida, 1-24.

[21] Wu KP, Ke JY, Chung CY, Chen CL, Hwang TL, Chou MY, et al. (2008). Relationship between unstimulated salivary flow rate and saliva composition of healthy children in Taiwan. *Chang Gung Med J*, 31,281-6.

[22] Farell E C, Kaplan A et al. (1984). Calcium. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton, 1051-1255.

[23] Kessler G. et al. (1964). *Clin Chem*,10(8), 686-706.

[24] Connerty H. V. et al. (1996).*Am J Clin Path*, 45(3), 200-296

[25] Farrell E C. (1984). Phosphorus. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton, 1072-1074.

[26] Daly J A. et al. (1972). *Clin Chem*, 18 (3): 263-265

[27] Tsai Al, Chen CY, Li LA, Hsiang CL, Hsu KH. (2006). Risk indicators for early childhood caries Taiwan. *Community Dent Oral Epidemiol*, 34:437-45

[28] Llena-Puy C. (2006). The role of saliva in maintaining oral health and as an aid to diagnosis. *Med OralPatol Oral Cir Bucal*, 11:449-55.

[29] Dodds MW, Johnson DA, Yeh CK. (2005). Health benefits of saliva: A review. *J Dent*, 33:223-33.

[30] Shahrabi M, Nikfarjam J, Alikhani A, Akhoundi N, Ashtiani M, Seraj B. (2008). A comparison of salivary calcium, phosphate, and alkaline phosphatase in children with severe, moderate caries, and caries-free in Tehran's kindergartens. *J Indian Soc Pedod Prev Dent*, 26(2):74-7.

[31] Jolly L, Shetty A. (2014). Calcium and inorganic phosphorous levels in stimulated and unstimulated saliva in early childhood caries-A comparative study. *J Academy Dent Edu*,1(2):5-11.

[32] Vogel JJ, Naujoks R, Brudevold F. (1965). The effective concentrations of calcium and inorganic orthophosphate in salivary secretions. *Arch oral biol*, 10(3):523-34.

[33] Bardow A, Hofer E, Nyvad B, ten Cate JM, Kirkeyby S, Moe D, Nauntofte B. (2005). Effect of saliva composition on experimental root caries. *Caries Res*, 39: 71-77.

[34] Bowen WH, Velez H, Aguila M, Velasquez H, Sierra LI, Gillespie G. (1977). The microbiology and biochemistry of plaque, saliva and drinking water from two communities with contrasting levels of caries in Colombia, *S.A. J Dent Res*, 55 (Sp Iss C): C32-C39.

[35] Bagherian A, Asadikaram G. Comparison of some salivary characteristics between children with and without early childhood caries. *Indian J Dent Res*, 23(5):628.

[36] Abbas MJ, Al-Hadithi HK, Mahmood MA, Hussein HM. (2020). Comparison of Some Salivary Characteristics in Iraqi Children with Early Childhood Caries (ECC) and Children without Early Childhood Caries. *Clin. Cosmet. Investig. Dent*, 12:541.

Table 1: Comparison of Salivary calcium levels between the groups

Groups	**N**	**Mean**	**SD**	**p-value**
Group I (Caries-	20	2.19	0.52	

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free)				0.018*
Group B (ECC)	20	2.69	1.11	
Group C (Severe ECC)	20	3.46	1.05	

p-value was evaluated using ANOVA. p-value < 0.05 – Significant

Table 2:Intergroup comparison of Salivary calcium levels

Groups	**Group I (Caries-free)**	**Group B (ECC)**	**Group C (Severe ECC)**
Group I (Caries-free)	-	0.46	0.05*
Group B (ECC)	0.46	-	0.17
Group C (Severe ECC)	<0.05*	0.17	-

p value < 0.05 – Significant – Post- hoc Tukey test

Table 3: Comparison of Salivary phosphorus levels between the groups

Groups	**N**	**Mean**	**SD**	**p-value**
Group I (Caries-free)	20	5.45	0.89	0.009*
Group B (ECC)	20	7.83	1.39	
Group	20	7.86	2.72	

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C (Severe ECC)				

p-value was evaluated using ANOVA. p-value < 0.05 – Significant *

Table 4:Intergroup comparison of Salivary Phosphorus levels

Groups	**Group I (Caries-free)**	**Group B (ECC)**	**Group C (Severe ECC)**
Group I (Caries-free)	-	<0.05*	0.05*
Group B (ECC)	<0.05*	-	0.99
Group C (Severe ECC)	<0.05*	0.99	-

p value < 0.05 – Significant – Post- hoc Tukey test